TITLE OF THE INVENTION

MAMMALIAN STAUFEN AND USE THEREOF.

FIELD OF THE INVENTION

The present invention relates to mammalian staufen, a double-stranded RNA-binding protein involved in mRNA transport and localization. The invention further relates to the demonstration of the association of a RNA-binding protein with the rough endoplasmic reticulum (RER), implicating staufen and related proteins in the transport of RNA to its site of translation. Broadly, the invention therefore relates to transport and translation of RNA. More specifically, the present invention relates to human and mouse staufen proteins and to the modulation of transport of RNA to the RER by these proteins. The present invention also relates to isolated nucleic acid molecules encoding mammalian staufen, as well as vectors and host cells harboring same. In addition, the present invention relates to screening assays for identifying modulators of staufen activity and to the identification of mutants thereof which abrogate their interaction with RER. Furthermore, the present invention relates to the use of the double-stranded RNA binding activity of staufen as a means to target proteins into virions. The invention in addition relates to the incorporation of staufen into RNA viruses and the use of overexpression of staufen to significantly decrease the infectivity thereof. More particularly, the present invention relates to a novel and broad class of molecules which can be used as carriers to target molecules into virions of RNA viruses and to decrease infectivity of a wide variety of RNA viruses including retroviruses.

BACKGROUND OF THE INVENTION

It is now believed that the cytoskeleton is widely used to transport mRNAs between their transcription and processing sites in the nucleus and their translation and degradation sites in the cytoplasm (Pachter, 1992; Bassell and Singer, 1997; Nakielny et al., 1997). One consequence of the interaction between mRNAs and the cytoskeleton is to promote differential localization and/or transport of mRNAs in subcellular compartments. Indeed, examples of mRNA targeting were observed in both germinal and somatic cells throughout the animal kingdom (Wilhelm and Vale, 1993; St Johnson, 1995; Steward, 1997). The universal use of this mechanism is also apparent when we consider the nature of the proteins which are coded by the transported mRNAs; asymmetric localization involving mRNAs coding for cytosolic, secreted, membrane-associated or cytoskeletal proteins have all been reported. Localization of mRNAs in the cytoplasm is now considered an essential step in the regulation of gene expression and an efficient way to unevenly distribute proteins in polarized cells. In general, it is believed that mRNA localization is used to determine and/or regulate local sites of translation (Rings et al., 1994; St Johnston, 1995; Steward, 1997). Indeed, ribosomes and many translational cofactors were found in association with the cytoskeletal elements, preventing both mRNAs and translation factors from being diluted by the cellular fluid (Pachter, 1992). Transport and local translation of specific mRNAs has been shown to play an important role in processes such as learning and memory (Martin et al., 1997), synaptic transmission (Crino and Eberwine, 1996; Kang and Schuman, 1996; Gazzaley et al., 1997; Steward, 1997; Tongiorgi et al., 1997), axis formation during development (reviewed in St Johnston,

1995), cell motility (Kislauskis et al., 1997), and asymetric cell division (Li et al., 1997; Long et al., 1997; Takizawa et al., 1997; Broadus et al., 1998).

The mechanisms underlying mRNA localization are not yet fully understood, mainly because of the lack of information on the principal constituents of the ribonucleoprotein complexes involved in this process. Nevertheless, it is known to involve both cis-acting signals in mRNA and trans-acting RNA-binding proteins which bind to this signal (St Johnston, 1995). The signals that allow mRNAs to be recognized as a target for transport and then to be localized have been mapped within their 3'-untranslated regions (Wilhelm and Vale, 1993; St Johnston, 1995). In contrast, the nature of the RNA-binding proteins is still obscure. Recently, a 68 kDa protein which binds the β-actin mRNA zipcode localization domain was isolated and its transcript was cloned from chicken cDNA libraries (Ross et al., 1997). This protein, which binds to microfilaments, contains an RNA-binding domain which shares strong sequence similarity with the RNP1 and RNP2 motifs. In addition, 69 kDa and 78 kDa proteins in Xenopus oocyte extracts have been shown to bind to the localization signal of Vg1 mRNA (Schwartz et al., 1992; Deshler et al. 1997). While the 69 kDa protein was shown to bind microtubules (Elisha et al.,1995), the 78 kDa Vera protein co-localized with a sub-domain of the smooth endoplasmic reticulum (Deshler et al., 1997). However, since these proteins have not yet been characterized, their nature and function in localization remain unclear.

Genetic and molecular studies have shown that the activity of the *staufen* gene product in *Drosophila* is necessary for the proper localization of *bicoid* and *oskar* mRNAs to the anterior and

posterior cytoplasm of oocytes, respectively, and of *prospero* mRNA in neuroblasts (St Johnston et al., 1989; Ephrussi et al., 1991; Kim-Ha et al., 1991; St Johnston et al., 1991; Broadus et al., 1997; Li et al., 1997). *Staufen* is a member of the double-stranded RNA-binding protein family, and contains three copies of a domain consisting of a 65- to 68-amino acid consensus sequence which is required to bind RNAs having double-stranded secondary structures, and two copies of a short-domain, which retains the last 21 amino acids at the C-terminal end of the complete motif (St Johnston et al., 1991; St Johnston et al., 1992). In vitro, it has been demonstrated that *staufen* binds directly to *bicoid* and *prospero* mRNAs (St Johnston et al., 1992; Li et al., 1997). However, since *staufen* seems to bind to any dsRNA in vitro, it is not clear whether or not it binds directly to these RNAs in vivo, or needs cellular co-factors which make up part of a larger ribonucleoprotein complex to localize each mRNA. Many experiments have demonstrated that the localization of *oskar*, *prospero* and *bicoid* mRNAs occurs through a multistep mechanism of active transport that is dependent on elements of the cytoskeleton (Erdelyi et al., 1995; Pokrywka and Stephenson, 1995; St Johnston, 1995; Tetzlaff et al., 1996; Broadus et al., 1997).

There thus remains a need to understand the mechanisms of mRNA transport in mammals and determine the nature of both the RNAs and proteins in the RNA/protein complexes. Recently, both Southern blot analysis of human DNA and fluorescent in situ hybridization (FISH) on human chromosomes in metaphase showed that the human gene is present as a single copy in the human genome and is localized in the middle of the long arm of chromosome 20 (DesGroseillers and Lemieux, 1996). The identification and characterization of human (or

another mammalian) *staufen* is desired as it could provide critical information in the transport, and proper localisation of mRNAs in subcellular compartments.

Staufen (Stau) was originally described as a dsRNA-binding protein in *Drosophila melanogaster* (1). It was further shown to specifically bind the 3' untranslated region of the mRNA for *bicoid* (2), a morphogen responsible for anterior body pattern formation in the early embryo. In *Drosophila*, Stau's principle function is to target mRNAs for localized translation (2, 3): it serves to localize *oskar* mRNA posteriorally (3) and anchors *bicoid* mRNA anteriorally in oocytes, and recently has been shown to localize *prospero* mRNA in neuroblasts (4). The human homologue (hStau) is hereinbelow further characterized and is shown to have several structural and functional domain similarities to its *Drosophila* counterpart (5).

A more thorough understanding of the structure-function relationship of mammalian *staufen* is needed to better understand its function in mammalian cells. There also remains a need to better understand the dsRNA-binding activity of mammalian *staufen* and to analyze the function and application thereof in cellular homoeostasy. In addition, this understanding could help characterize the important molecular determinants of staufen from lower eukaryotes.

It would be highly desirable to be provided with means to target molecules to RNA viruses, including retroviruses, such as HIV virions. It would also be desirable to be provided wth means to target molecules into such viruses and affect their structural organization and/or functional integrity and/or morphogenesis.

It would also be highly desirable to be provided with a protein, fragment or derivative thereof which permits the development of chimeric molecules that can be specifically targeted into RNA viruses in general, and more particularly retroviruses, including antiviruses such as HIV. Such chimeric molecules could be used for the treatment of RNA virus infections, retroviral infections and lentiviral infections.

It would also be highly desirable to be provided with a therapeutic agent which permits targeting of chimeric molecules into RNA virions, as a treatment for diseases caused by such virions.

It would also be highly desirable to be provided with the identification of novel molecular determinants responsible for the incorporation of proteins into virions via their interaction with genomic RNA, for RNA genome incorporation into RNA viruses, as well as the identification of molecular determinants involved in the targeting of RNA molecules to the RER.

It would also be highly desirable to be provided with means to target RNA molecules to the RER.

It would also be very desirable to be provided with therapeutic agent molecules which interfere with the molecular determinant responsible for RNA genome incorporation into RNA virions as well as agents which interfere with the targeting of RNA molecules to the RER as such agents could have therapeutic utility for the treatment of diseases including viral diseases.

It would further be highly desirable to be provided with an assay which enables the screening and identification of molecules which modulate the interaction between the molecular determinant responsible for RNA genome incorporation into RNA virions. As well, it

would be highly desirable to be provided with an assay which enables the screening and identification of molecules which modulate the targeting of RNA molecules to the RER.

It would in addition be highly desirable to be provided with a method for screening and identifying molecules which act as modulating agents of RNA genome incorporation into RNA virions and as well as a method for screening and identifying molecules which act as modulating agents for the targeting of RNA molecules to RER.

The present invention seeks to meet these and other needs.

The present description refers to a number of documents, the content of which is herein incorporated by reference.

The human homologue of the double-stranded RNA (dsRNA)-binding protein, *Staufen*, is shown herein to be incorporated into HIV-1 virions, and this correlates with HIV-1 genomic RNA encapsidation. hStau is incorporated into clinical isolates of HIV-1, and several other retroviruses including HIV-2 and murine leukemia virus, and non-retroviral RNA viruses such as Reovirus, but is not detectable in DNA viruses. When hStau is overexpressed, a corresponding increase of hStau in virions is observed. Strikingly, this increase in hStau incorporation into HIV-1 is accompanied by a dramatic impairment of HIV-1 infectivity. This is the first demonstration of a dsRNA-binding protein within HIV-1 particles. This novel and unexpected finding may have important implications not only in retroviral genome sorting, assembly and infectivity, but also in RNA virus therapy in general, retrovirus therapy and more particularly HIV-1 therapy.

The invention concerns in general mammalian *staufen* and more particularly the sequence of the human and mouse staufen proteins and nucleic acid molecules encoding same.

The present invention further relates to the demonstration that staufen binds both dsRNA and tubulin in vitro via specific binding domains. Further, the invention relates to the localization of *staufen* in the cytoplasm in association with the rough endoplasmic reticulum, implicating this protein in the targeting of RNA to its site of translation.

More particularly, the present invention provides isolated polypeptides having the amino acid sequences shown in Figures 1A, 1B, 1C, 1D and Figure 1'.

The present invention further relates to isolated nucleic acid molecules comprising polynucleotides which encode a staufen polypeptide and more particularly a mammalian staufen polypeptide. More paticularly, the present invention relates to isolated nucleic acid molecules encoding the staufen polypeptides having the amino acid sequences shown in Figures 1A, 1B, 1C and 1'.

The invention in addition relates to recombinant vectors harboring the isolated nucleic acid molecules of the present invention. More particularly, the invention relates to expression vectors which express the staufen polypeptides of the present invention and more particularly mammalian staufen. The present invention further relates to host cells containing such recombinant vectors or expression vectors, to methods of making such host cells, and to methods of making such vectors.

Further, the present invention provides screening assays and methods for identifying modulators of staufen activity and especially of mammalian staufen activity. More particularly, the present invention relates to assays and methods for screening and identifying compounds which can enhance or inhibit the RNA virion incorporation ability of staufen and especially mammalian staufen. In one particular embodiment of the present invention, the screening assay for identifying modulators of staufen's incorporation ability comprises contacting cells or extracts containing staufen and a candidate compound, assaying a cellular response or biological function of staufen such as virion incorporation or RER targeting, for example, wherein the potential modulating compound is selected when the cellular response or staufen's biological activity in the presence of the candidate compound is measurably different than in the absence thereof.

In addition, the present invention relates to methods for treating an animal (such as a human) infected with a RNA virus, which comprises administration thereto of a composition comprising a therapeutically effective amount of staufen (such as mammalian staufen) polypeptide, and /or staufen nucleic acid molecule encoding same, and/or modulators of staufen activity. In one embodiment, the present invention relates to an administration of a recombinant staufen molecule having an additional antiviral activity (i.e. RNAse or protease activity).

The invention further relates to the use of polypeptides and nucleic acid molecules encoding same of the present invention to target molecules into virions of RNA viruses. In a particular embodiment, such targeting finds utility for example, in packaging cell lines. In a

particular embodiment, staufen is used as a carrier for virion targeting and is part of a fusion protein.

In accordance with the present invention, there is therefore provided, an isolated mammalian staufen protein exhibiting homology to mammalian staufen as well as lower eukaryotic staufen.

In accordance with the present invention, there is also provided, an isolated nucleic acid molecule comprising a polynucleotide sequence encoding mammalian staufen.

In accordance with another aspect of the present invention, there is provided, an isolated nucleic acid molecule comprising a polynucleotide sequence which hybridizes under stringent conditions to a polynucleotide sequence encoding mammalian staufen or to a sequence which is complementary thereto.

In accordance with yet another aspect of the present invention, there is provided a method of constructing a recombinant vector which comprises inserting an isolated nucleic acid molecule encoding mammalian staufen (or a derivative thereof) into a vector. In addition, there is also provided a recombinant vector harboring an isolated nucleic acid molecule encoding a *C. elegans* staufen or fragments or derivatives thereof. In addition, there is provided recombinant vectors harboring an isolated nucleic acid molecule encoding the molecular determinant of a mammalian or lower eukaryotic staufen, which is responsible for incorporation into RNA virions.

In accordance with a further aspect of the present invention, there is provided a method for making a recombinant cell comprising introducing thereinto a recombinant vector harboring a nucleic acid sequence encoding a staufen of the present invention.

In accordance with an additional aspect of the present invention, there is provided an antibody which recognizes specifically a staufen polypeptide or derivative thereof of the present invention.

The mammalian staufen polypeptides and nucleic acid molecules of the instant invention have been isolated from human and mouse. Nevertheless, it will be clear to the person of ordinary skill that the present invention should not be so limited. Indeed, using the teachings of the present invention and those of the art, homologues of hStau and mStau can be identified and isolated from other animal species. Non-limiting examples thereof include monkey, mouse, rat, rabbit, and frog. The significant identity between the human and mouse Staufen protein validates this contention.

The conservation of staufen between mammals and lower eukaryotes (*Drosphila* and *C. elegans*) further supports this notion. In addition, it suggests that certain embodiments of the present invention could be carried out using lower eukaryotic staufen or fragments or derivatives thereof.

The invention further relates to the morphogenesis RNA virions and more particularly of HIV virions and especially to the packaging of RNA genomes into RNA viruses.

The present invention further provides means to target molecules to RNA virions. In one particular embodiment, the present invention relates to such means to affect the morphogenesis of such RNA virions, thereby reducing infectivity thereof. In a particularly preferred embodiment, the present invention relates to a mammalian staufen protein which upon incorporation into HIV-1 virions significantly decreases the infectivity thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Having thus generally described the invention, reference will now be made to the accompanying drawings, showing by way of illustration a preferred embodiment thereof, and in which:

Figure 1A shows an amino acid sequences of the human staufen cDNAs. Alignment of the two cDNAs with the translation of the putative protein sequences. The numbers refer to the sequence of the short cDNA. The positions of the 4 dsRNA-binding consensus domains (RBD1 to RBD4) and of the tubulin-binding domain (TBD) are indicated between brackets above the sequence. The sequences were deposited in the GenBank database under accession numbers AF061938 and AF061939.

Figure 1B is similar to Figure 1 but shows the alternative splicing which occurs in the human *staufen* gene and gives rise to 4 alternatively spliced transcripts. These 4 transcripts give rise to the two proteins as described in Figure 1 and in the text below. Of note, transcripts T2 and T3 refer to transcripts T1 and T2 of Figure 1A, respectively.

Figure 1C shows the nucleic acid and predicted amino acid sequence of mouse *staufen*.

Figure 1D shows an alignment of the mouse and human *staufen*, highlighting the significant conservation of the protein during evolution. As per Figure 1, the 4 dsRNA binding domains (RBD) and tubulin-binding domains are highlighted.

Figure 1' shows an alignment between phylogenetically different *staufen* proteins of *Drosophila*, *C. elegans* and human. This alignment permits the elaboration of a consensus sequence for *staufen*.

As shown in Figure 1B, T1, T2 and T4 give rise to the short protein of 55 kDa while T3 gives rise to the 63 kDa protein.

Figure 2 shows the characterization of the hStau mRNA and proteins. A) Northern blot analysis of hStau expression in human tissues. A Human Multiple Tissues Northern Blot (Clontech) was hybridized with the 1.2 kbp BamHI fragment of hStau cDNA. Lane 1, brain; lane 2, pancreas; lane 3, heart; lane 4, skeletal muscles; lane 5, liver; lane 6, placenta; lane 7, lung; lane 8, kidney). B) Western blot experiment with anti-hStau antibodies. Lane 1, HeLa cell extracts; lane 2, HEK 293 cell extracts. C) HEK cells were transfected with cDNAs coding for either the short (lane 2) or the long (lane 3) hStau isoforms, lysed and analysed by western blotting using the anti-hStau antibodies. Mock-transfected cells are shown in lane 1. D) Schematic representation of the *Drosophila* (accession number M69111), mammalian and C. elegans (accession number U67949) staufen proteins. The human protein P1 has an insertion of 81 amino acids at its N-terminal extremity, as compared to protein P2. Large open and black boxes represent the full-length and short dsRNA-binding domains, respectively. Small boxes and lines are regions of high and low sequence similarity, respectively. The hatched boxes indicate the position of the region which is similar to the MAP1B microtubule-binding domain. The percentage of identity between the domains of the human and invertebrate proteins is indicated.

Figure 3 shows an RNA-binding assay. A) Bacterially expressed his/hStau (lanes S) and his/NEP (lane N) fusion proteins or B) bacterially-expressed MBP/mStau (lanes S) or MBP/aminopeptidase fusion proteins (lane A), were electrophoresed on a polyacrylamide gel, transferred to nitrocellulose, and incubated with [32P]labeled nucleic

acids, in the presence or absence of cold competitors, as indicated below each gel. After extensive washing, binding was detected by autoradiography.

Figure 4 shows a tubulin-binding assay. Bacterially expressed MBP/hStau (lanes S) or MBP/aminopeptidase (lanes A) fusion proteins were electrophoresed on SDS-polyacrylamide gels, transferred to nitrocellulose, and incubated with tubulin or actin. After extensive washing, tubulin and actin were detected with monoclonal anti-tubulin or anti-actin antibodies, respectively. As controls, the same experiments were also performed in the absence of either tubulin or anti-tubulin antibodies. Purified actin was also loaded on the gel as control (lane C).

Figure 5 shows a molecular mapping of the dsRNA- and tubulin-binding domains. Bacterially expressed MBP/mStau (lanes 1), MBP/mStau deletion mutants (lanes 2-7) or MBP/aminopeptidase (lanes C) fusion proteins were electrophoresed on a polyacrylamide gel, transferred to nitrocellulose, and incubated either with [32P]labeled 3'-UTR *bicoid* RNA (A) or tubulin and anti-tubulin antibodies (B), and revealed as described above. C) Schematic representation of the mutant proteins. Their RNA- and tubulin-binding responses are indicated.

Figure 6 shows a subcellular localization of the GFP/hStau fusion proteins. COS7 cells were transfected with cDNAs coding for either the hStau/GFP (A, B) or TBD/GFP (C) fusion proteins, or GFP alone (D). Untreated (A, C, D) or Triton X-100 treated (B) cells were fixed and visualized by autofluorescence. Bar = 20 mm.

Figure 7 shows a co-localization of hStau with markers of the rough endoplasmic reticulum (RER) using confocal microscopy. A cDNA coding for an hStau/HA fusion protein was transfected into COS7

cells. Triton X-100 treated cells were fixed and double-labeled with anti-HA (B) and anti-calreticulin (A) or anti-HA (E) and anti-calnexin (D). Anti-HA was detected with Texas Red-coupled anti-mouse IgG antibodies using the Texas Red channel, whereas anti-calreticulin and anti-calnexin were detected with fluorescein-conjugated anti-rabbit IgG antibodies, using the fluorescein channel. C and F are the superposition of A-B and D-E, respectively. No overlap was observed between the fluorescein and Texas Red channels. Bar = 10 mm.

Figure 8. A. Northwestern analysis of hStau TAR RNA-binding. Extracts of bacteria expressing either histidine(his)-tagged hStau (lane 1) or his-NEP (neutral endopeptidase, lane 2) fusion proteins were electrophoresed on a polyacrylamide gel (PAGE), transferred to nitrocellulose and incubated for 2 h with a uniformly [^{32}P]labelled TAR(1-80) RNA in 50 mM NaCl, 10 mM $MgCl_2$, 10 mM Hepes, pH 8.0, 0.1 mM EDTA, 1 mM DTT, 0.25% milk. After extensive washing, the membrane was exposed to autoradiographic film. B. Sucrose density gradient analysis of hStau in HIV-1. 50 X 10^6 cpm of microfiltered and ultracentrifuged virus HxBru was layered onto a continuous 20-60% sucrose gradient, ultracentrifuged at 136 000 x *g* for 16 h. 16-0.7 mL fractions were collected and RT activity was measured by standard assay. Each fraction was subsequently diluted to 20% sucrose and centrifuged at 136 000 x *g* for 1 hr to pellet virus particles. After rinsing, the virus pellet was resuspended in PBS and 2X Laemmli loading buffer was added before loading onto a 10% PAGE. The proteins were transferred to nitrocellulose and probed with a rabbit anti-hStau antibody. hStau was visualized using the enhanced chemiluminescence (ECL) kit (Amersham, Mississauga, ON). C. Subtilisin protease resistance assay.

Subtilisin assays were performed essentially according to Ott *et al.*, (9, 10) with minor modifications. 70 x 10^6 cpm of pelletted virus preparations were treated (+) or mock treated (-) with 1 mg/mL subtilisin (Boehringer Mannheim, Montreal, PQ) in 10 mM Tris-HCl, pH 8, 1 mM $CaCl_2$, containing 1.5 mg/mL bovine serum albumin (ICN Biochemicals, Montreal, PQ) for 24 h at 37°C. Virus was then pelletted as above and resuspended in PBS, and made to 1X Laemmli and then loaded onto PAGE followed by Western blotting. The blot was sequentially probed with anti-gp120 (32), a mouse monoclonal antibody #3H11-C1 to p17 (33), a human patient's serum (#162) to reveal p24, and anti-hStau. D. hStau incorporation into virus particles from clinical isolates and the retroviruses HIV-2, MLV, and CasBr. 293T cells were transfected with proviral constructs encoding HIV-1, HIV-2 (ROD), MLV (kindly provided by Dr. Guy Lemay, University of Montreal) and CasBr retroviruses (11). Virus (passage # 2) was also harvested following infection of MT4 cells with two T-tropic viral clinical isolates (T1 & T2; a kind gift from Dr. Mark Wainberg, McGill AIDS Center). 10 x 10^6 RT cpm (HIV-1, HIV-2 and MLV) were loaded onto gels and incorporated hStau was assessed by Western blotting. The 55 and 63kDa hStau species are due to translation initiation from alternatively spliced transcripts (5). Longer exposures reveal both species in all lanes. E. hStau is the only TAR-binding protein to be virion incorporated. Three sets of 25 000 293T cell equivalents (C) and 50 ng p24 virus equivalents (V) were run in parallel on 10% PAGE and each of three blots was probed with antibodies to hStau, TRBP (kindly provided by Dr. Sundararajan Venkatesan, NIAID), and PKR (kindly provided by Dr. Antonis Koromilas, McGill University). For the assessment of Tat in virus particles, 293T cells were transfected with

pNL4.3 and at 48 h postinfection (p.i.) cells were lysed in Laemmli buffer and 25 000 cell equivalents were run in parallel with 50 ng p24. An amino-terminal anti-peptide Tat antibody was used for Western blot analysis. Antigens were revealed by ECL and are indicated by bold arrowheads. TRBP, PKR and Tat were undetectable in virion preparations in longer exposures of Western blots.

Figure 9 shows a localization of hStau in cotransfected 293T cells by confocal laser scanning microscopy. 293T cells were cotransfected with pNL4.3 and a plasmid encoding a HA-tagged hStau (5). 36 h posttransfection, cells were trypsinized and plated on glass slides and allowed to grow for 12 h. After washing, cells were fixed with acetone:methanol (50:50) and allowed to dry. Indirect immunofluorescence was performed using a mouse anti-HA monoclonal (12CA5, Boehringer Mannheim) and a rabbit anti-p24 (34). Texas Red- and fluorescein-conjugated secondary antibodies were employed to reveal p24 and HA-hStau, respectively. Confocal laser scanning microscopy was performed using a Zeiss LSM410 microscope with excitation wavelengths of 488 nm and 568 nm for fluorescein and Texas Red, respectively. Emission filters for fluorescein and Texas Red were BP515-540, and BP575-640, respectively. p24 (A), hStau (B), and superimposed images (C) are presented. The yellow regions indicate colocalization of p24 and hStau (mostly at the cell periphery). A representative cell is shown.

Figure 10 shows hStau incorporation correlating with genomic RNA encapsidation in HIV-1 particles. Proviral DNAs [(wildtype, NC (14, 15), *vpr-* or *vpr+* (6) and *psi* mutants (16)] were transfected into 293T cells and equal quantities of virus were loaded onto 12% PAGE and

probed with anti-hStau (A) and anti-p17 (B; 33) antisera and antigens were revealed by ECL. In C, RNA was isolated from equal quantities of virus using an NP-40 lysis method (35) and probed with a [^{32}P]-labelled probe to the Gag mRNA leader (6). Lane 1, pNL4.3; lane 2, HxBru; lane 3, ^{28}C/^{49}C-S NC; lane 4, ^{15}C/^{18}C-S NC; lane 5, ^{36}C/^{39}C-S NC; lane 6 delta ^{14}K-^{50}T NC; lane 7, *psi* signal mutant; lane 8, HxBru Vpr- provirus; lane 9, HxBru Vpr+ provirus.

Figure 11 shows overexpression of hStau causing a decrease of infectivity of HIV-1 particles. 10 μg pNL4.3 was transfected into 293T cells with or without an expression plasmid encoding HA-hStau at a 1:1.3 molar basis (or KS DNA carrier). A, Virus was prepared from mock, pNL4.3 and pNL4.3+hStau transfected cells and used in Western blot analysis using equal quantities of p24 in each lane. For infectivity assays, equal quantities of p24 were used to infect MAGI and BF-24 indicator cells and infectivity was quantitated at 48 h p.i. by colorimetric and CAT activity assays, respectively. B, BF-24 cells were washed extensively and lysed by freeze-thaw in 0.25 M Tris, pH 7.5, followed by heat inactivation. CAT activity in cells was determined by standard assay by thin layer chromatography (a representative result is shown here). C, The data shown are the means and standard errors of the means (S.E.M.) from three independent infectivity assays in BF-24 cells. Relative CAT activity (compared to the pNL4.3 lane which is set to 1) was calculated by phosphoimager analysis using the Molecular Dynamics ImageQuant software. MAGI assay results conferred with those from BF-24 assays revealing a 4-fold (±0.3, S.E.M.) reduction in the number of blue β-galactosidase-positve cells 48 h p.i. (7).

Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of preferred embodiments with reference to the accompanying drawing which is exemplary and should not be interpreted as limiting the scope of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention therefore relates to *staufen*, a double-stranded RNA-binding protein which binds dsRNA via each of two full-length dsRNA-binding domains and tubulin via a region similar to the microtubule-binding domain of MAP1B. Immunofluorescent double-labeling of transfected mammalian cells revealed that Stau is localized to the rough endoplasmic reticulum (RER), implicating this RNA-binding protein in mRNA targeting to the RER. These results are the first demonstration of the association of an RNA-binding protein with the RER, implicating this class of proteins in the transport of RNA to its site of translation.

The human homologue of the double-stranded RNA (dsRNA)-binding protein, *Staufen*, is shown herein to be incorporated into HIV-1 virions, and this correlates with HIV-1 genomic RNA encapsidation. hStau is incorporated into clinical isolates of HIV-1, and several other retroviruses including HIV-2 and murine leukemia virus, and non-retroviral RNA viruses such as Reovirus, but is not detectable in DNA viruses. Experiments with poliovirus are underway and are expected to further demonstrate the role of staufen during the life cycle of RNA viruses in general. When hStau is overexpressed, a corresponding increase of hStau in virions is observed. Strikingly, however, this increase in hStau

incorporation into HIV-1 is accompanied by a dramatic impairment of HIV-1 infectivity. This is the first demonstration of a dsRNA-binding protein within HIV-1 particles. This novel and unexpected finding may have important implications not only in retroviral genome sorting, assembly and infectivity, but also in RNA virus therapy in general and more in particularly HIV-1 therapy.

Nucleotide sequences are presented herein by single strand, in the 5' to 3' direction, from left to right, using the one letter nucleotide symbols as commonly used in the art and in accordance with the recommendations of the IUPAC-IUB Biochemical Nomenclature Commission.

Unless defined otherwise, the scientific and technological terms and nomenclature used herein have the same meaning as commonly understood by a person of ordinary skill to which this invention pertains. Generally, the procedures for cell cultures, infection, molecular biology methods and the like are common methods used in the art. Such standard techniques can be found in reference manuals such as for example Sambrook et al. (1989, Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratories) and Ausubel et al. (1994, Current Protocols in Molecular Biology, Wiley, New York).

The present description refers to a number of routinely used recombinant DNA (rDNA) technology terms. Nevertheless, definitions of selected examples of such rDNA terms are provided for clarity and consistency.

As used herein, "nucleic acid molecule", refers to a polymer of nucleotides. Non-limiting examples thereof include DNA (i.e. genomic DNA, cDNA) and RNA molecules (i.e. mRNA). The nucleic acid

molecule can be obtained by cloning techniques or synthesized. DNA can be double-stranded or single stranded (coding strand or non-coding strand [antisense]).

The term "isolated nucleic acid molecule" refers to a nucleic acid molecule purified from its natural environment. Non-limiting examples of an isolated nucleic acid molecule is a DNA sequence inserted into a vector, and a partially purified polynucleotide sequence in solution.

The term "recombinant DNA" as known in the art refers to a DNA molecule resulting from the joining of DNA segments. This is often referred to as genetic engineering.

The term "DNA segment", is used herein, to refer to a DNA molecule comprising a linear stretch or sequence of nucleotides. This sequence when read in accordance with the genetic code, can encode a linear stretch or sequence of amino acids which can be referred to as a polypeptide, protein, protein fragment and the like.

The terminology "amplification pair" refers herein to a pair of oligonucleotides (oligos) of the present invention, which are selected to be used together in amplifying a selected nucleic acid sequence by one of a number of types of amplification processes, preferably a polymerase chain reaction. Other types of amplification processes include ligase chain reaction, strand displacement amplification, or nucleic acid sequence-based amplification, as explained in greater detail below. As commonly known in the art, the oligos are designed to bind to a complementary sequence under selected conditions. For example, homologs of human or mouse staufen could be isolated using an amplification method such as PCR with an amplification

pair designed by comparing the homology of the human and mouse sequences.

The nucleic acid (i.e. DNA or RNA) for practicing the present invention may be obtained according to well known methods.

As used herein, the term "physiologically relevant" is meant to describe interactions which can modulate transcription of a gene in its natural setting.

Oligonucleotide probes or primers of the present invention may be of any suitable length, depending on the particular assay format and the particular needs and targeted genomes employed. In general, the oligonucleotide probes or primers are at least 12 nucleotides in length, preferably between 15 and 24 molecules, and they may be adapted to be especially suited to a chosen nucleic acid amplification system. As commonly known in the art, the oligonucleotide probes and primers can be designed by taking into consideration the melting point of hydrizidation thereof with its targeted sequence (see below and in Sambrook et al., 1989, Molecular Cloning - A Laboratory Manual, 2nd Edition, CSH Laboratories; Ausubel et al., 1989, in Current Protocols in Molecular Biology, John Wiley & Sons Inc., N.Y.).

The terms "DNA oligonucleotide", or "DNA molecule" or "DNA sequence" refer to a molecule comprised of the deoxyribonucleotides adenine (A), guanine (G), thymine (T) and/or cytosine (C). Oligonucleotide or DNA can be found in linear DNA molecules or fragments, viruses, plasmids, vectors, chromosomes or synthetically derived DNA.

"Nucleic acid hybridization" refers generally to the hybridization of two single-stranded nucleic acid molecules having

complementary base sequences, which under appropriate conditions will form a thermodynamically favored double-stranded structure. Examples of hybridization conditions can be found in the two laboratory manuals referred above (Sambrook et al., 1989, *supra* and Ausubel et al., 1989, *supra*) and are commonly known in the art. In the case of a hybridization to a nitrocellulose filter, as for example in the well known Southern blotting procedure, a nitrocellulose filter can be incubated overnight at 65°C with a labeled probe in a solution containing high salt (5 x SSC or 5 x SSPE), 5 x Denhardt's solution, 1% SDS, and 100 µg/ml denatured carried DNA (i.e. salmon sperm DNA). The non-specifically binding probe can then be washed off the filter by several washes in 0.2 x SSC/0.1% SDS at a temperature which is selected in view of the desired stringency: room temperature (low stringency), 42°C (moderate stringency) or 65°C (high stringency). The selected temperature is based on the melting temperature (Tm) of the DNA hybrid. Of course, RNA-DNA hybrids can also be formed and detected. In such cases, the conditions of hybridization and washing can be adapted according to well known methods by the person of ordinary skill. Stringent conditions will be preferably used (Sambrook et al.,1989, *supra*). As well known in the art other stringent hybridization conditions can be used (i.e. 42°C in the presence of 50% of formamide).

Probes of the invention can be utilized with naturally occurring sugar-phosphate backbones as well as modified backbones including phosphorothioates, dithionates, alkyl phosphonates and α-nucleotides and the like. Modified sugar-phosphate backbones are generally taught by Miller, 1988 (Ann. Reports Med. Chem. 23:295) and Moran et al., 1987 (Nucl. Acids Res., 14:5019). Probes of the invention

can be constructed of either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), and preferably of DNA.

The types of detection methods in which probes can be used include Southern blots (DNA detection), dot or slot blots (DNA, RNA), and Northern blots (RNA detection). labeled proteins could also be used to detect a particular nucleic acid sequence to which it binds. Other detection methods include kits containing probes on a dipstick setup and the like.

Although the present invention is not specifically dependent on the use of a label for the detection of a particular nucleic acid sequence, such a label might be beneficial, by increasing the sensitivity of the detection. Furthermore, it enables automation. Probes can be labeled according to numerous well known methods (Sambrook et al., 1989, *supra*). Non-limiting examples of labels include ^{3}H, ^{14}C, ^{32}P, and ^{35}S. Non-limiting examples of detectable markers include ligands, fluorophores, chemiluminescent agents, enzymes, and antibodies. Other detectable markers for use with probes, which can enable an increase in sensitivity of the method of the invention, include biotin and radionucleotides. It will become evident to the person of ordinary skill that the choice of a particular label dictates the manner in which it is bound to the probe.

As commonly known, radioactive nucleotides can be incorporated into probes of the invention by several methods. Non-limiting examples thereof include kinasing the 5' ends of the probes using gamma ^{32}P ATP and polynucleotide kinase, using the Klenow fragment of Pol I of *E. coli* in the presence of radioactive dNTP (i.e. uniformly labeled DNA probe using random oligonucleotide primers in low-melt gels), using the

SP6/T7 system to transcribe a DNA segment in the presence of one or more radioactive NTP, and the like.

As used herein, "oligonucleotides" or "oligos" define a molecule having two or more nucleotides (ribo or deoxyribonucleotides). The size of the oligo will be dictated by the particular situation and ultimately on the particular use thereof and adapted accordingly by the person of ordinary skill. An oligonucleotide can be synthetised chemically or derived by cloning according to well known methods.

As used herein, a "primer" defines an oligonucleotide which is capable of annealing to a target sequence, thereby creating a double stranded region which can serve as an initiation point for DNA synthesis under suitable conditions.

Amplification of a selected, or target, nucleic acid sequence may be carried out by a number of suitable methods. See generally Kwoh et al., 1990, Am. Biotechnol. Lab. 8:14-25. Numerous amplification techniques have been described and can be readily adapted to suit particular needs of a person of ordinary skill. Non-limiting examples of amplification techniques include polymerase chain reaction (PCR), ligase chain reaction (LCR), strand displacement amplification (SDA), transcription-based amplification, the Qβ replicase system and NASBA (Kwoh et al., 1989, Proc. Natl. Acad. Sci. USA 86, 1173-1177; Lizardi et al., 1988, BioTechnology 6:1197-1202; Malek et al., 1994, Methods Mol. Biol., 28:253-260; and Sambrook et al., 1989, *supra*). Preferably, amplification will be carried out using PCR.

Polymerase chain reaction (PCR) is carried out in accordance with known techniques. See, e.g., U.S. Pat. Nos. 4,683,195; 4,683,202; 4,800,159; and 4,965,188 (the disclosures of all three U.S.

Patent are incorporated herein by reference). In general, PCR involves, a treatment of a nucleic acid sample (e.g., in the presence of a heat stable DNA polymerase) under hybridizing conditions, with one oligonucleotide primer for each strand of the specific sequence to be detected. An extension product of each primer which is synthesized is complementary to each of the two nucleic acid strands, with the primers sufficiently complementary to each strand of the specific sequence to hybridize therewith. The extension product synthesized from each primer can also serve as a template for further synthesis of extension products using the same primers. Following a sufficient number of rounds of synthesis of extension products, the sample is analysed to assess whether the sequence or sequences to be detected are present. Detection of the amplified sequence may be carried out by visualization following EtBr staining of the DNA following gel electrophores, or using a detectable label in accordance with known techniques, and the like. For a review on PCR techniques (see PCR Protocols, A Guide to Methods and Amplifications, Michael et al. Eds, Acad. Press, 1990).

Ligase chain reaction (LCR) is carried out in accordance with known techniques (Weiss, 1991, Science 254:1292). Adaptation of the protocol to meet the desired needs can be carried out by a person of ordinary skill. Strand displacement amplification (SDA) is also carried out in accordance with known techniques or adaptations thereof to meet the particular needs (Walker et al., 1992, Proc. Natl. Acad. Sci. USA 89:392-396; and ibid., 1992, Nucleic Acids Res. 20:1691-1696.

As used herein, the term "gene" is well known in the art and relates to a nucleic acid sequence defining a single protein or polypeptide. A "structural gene" defines a DNA sequence which is

transcribed into RNA and translated into a protein having a specific amino acid sequence thereby giving rise the a specific polypeptide or protein. It will readily recognized by the person of ordinary skill, that the nucleic acid sequence of the present invention can be incorporated into anyone of numerous established kit formats which are well known in the art.

A "heterologous" (i.e. a heterologous gene) region of a DNA molecule is a subsegment segment of DNA within a larger segment that is not found in association therewith in nature. The term "heterologous" can be similarly used to define two polypeptidic segments not joined together in nature. Non-limiting examples of heterologous genes include reporter genes such as luciferase, chloramphenicol acetyl transferase, β-galactosidase, and the like which can be juxtaposed or joined to heterologous control regions or to heterologous polypeptides.

The term "vector" is commonly known in the art and defines a plasmid DNA, phage DNA, viral DNA and the like, which can serve as a DNA vehicle into which DNA of the present invention can be cloned. Numerous types of vectors exist and are well known in the art.

The term "expression" defines the process by which a structural gene is transcribed into mRNA (transcription), the mRNA is then being translated (translation) into one polypeptide (or protein) or more.

The terminology "expression vector" defines a vector or vehicle as described above but designed to enable the expression of an inserted sequence following transformation into a host. The cloned gene (inserted sequence) is usually placed under the control of control element sequences such as promoter sequences. The placing of a cloned gene under such control sequences is often refered to as being operably linked to control elements or sequences.

Operably linked sequences may also include two segments that are transcribed onto the same RNA transcript. Thus, two sequences, such as a promoter and a "reporter sequence" are operably linked if transcription commencing in the promoter will produce an RNA transcript of the reporter sequence. In order to be "operably linked" it is not necessary that two sequences be immediately adjacent to one another.

Expression control sequences will vary depending on whether the vector is designed to express the operably linked gene in a prokaryotic or eukaryotic host or both (shuttle vectors) and can additionally contain transcriptional elements such as enhancer elements, termination sequences, tissue-specificity elements, and/or translational initiation and termination sites. Typically, expression vectors are prokaryote specific or eukaryote specific although shuttle vectors are also widely available.

Prokaryotic expression are useful for the preparation of large quantities of the protein encoded by the DNA sequence of interest. This protein can be purified according to standard protocols that take advantage of the intrinsic properties thereof, such as size and charge (i.e. SDS gel electrophoresis, gel filtration, centrifugation, ion exchange chromatography...). In addition, the protein of interest can be purified via affinity chromatography using polyclonal or monoclonal antibodies. The purified protein can be used for therapeutic applications.

The DNA construct can be a vector comprising a promoter that is operably linked to an oligonucleotide sequence of the present invention, which is in turn, operably linked to a heterologous gene, such as the gene for the luciferase reporter molecule. "Promoter"

refers to a DNA regulatory region capable of binding directly or indirectly to RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of the present invention, the promoter is bound at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter will be found a transcription initiation site (conveniently defined by mapping with S1 nuclease), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CCAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

As used herein, the designation "functional derivative" denotes, in the context of a functional derivative of a sequence whether an nucleic acid or amino acid sequence, a molecule that retains a biological activity (either function or structural) that is substantially similar to that of the original sequence. This functional derivative or equivalent may be a natural derivatives or may be prepared synthetically. Such derivatives include amino acid sequences having substitutions, deletions, or additions of one or more amino acids, provided that the biological activity of the protein is conserved. The same applies to derivatives of nucleic acid sequences which can have substitutions, deletions, or additions of one or more nucleotides, provided that the biological activity of the sequence is generally maintained. When relating to a protein sequence, the substituting amino acid as chemico-physical properties which are similar to that of the substituted amino acid. The similar

chemico-physical properties include, similarities in charge, bulkiness, hydrophobicity, hydrophylicity and the like. The term "functional derivatives" is intended to include "functional fragments", "functional segments", "functional variants", "functional analogs" or "functional chemical derivatives" of the subject matter of the present invention. "Fragments" of the nucleic acid molecules according to the present invention refer to such molecules having at least 12 nt, more particularly at least 18 nt, and even more preferably at least 24 nt which have utility as diagnostic probes and/or primers. It will become apparent to the person of ordinary skill that larger fragments of 100 nt, 1000 nt, 2000 nt and more also find utility in accordance with the present invention.

The term "at least 24 nt" is meant to refer to 24 contiguous nt of a chosen sequence such as shown for example in Figure 1A, 1B, 1C, 1D and 1'.

The term "functional variant" refers herein to a protein or nucleic acid molecule which is substantially similar in structure and biological activity to the protein or nucleic acid of the present invention.

The functional derivatives of the present invention can be synthesized chemically or produced through recombinant DNA technology, all these methods are well known in the art.

The term "molecule" is used herein in a broad sense and is intended to include natural molecules, synthetic molecules, and mixture of natural and synthetic molecules. The term "molecule" is also meant to cover a mixture of more than one molecule such as for example pools or libraries of molecules. Non-limiting examples of molecules include chemicals, biological macromolecules, cell extracts and the like. The term

"compound" is used herein interchangeably with molecule and is similarly defined.

Nucleic acid fragments in accordance with the present invention include epitope-encoding portions of the polypeptides of the invention. Such portions can be identified by the person of ordinary skill using the nucleic acid sequences of the present invention in accordance with well known methods. Such epitopes are useful in raising antibodies that are specific to the polypeptides of the present invention. The invention also provides nucleic acid molecules which comprise polynucleotide sequences capable of hybridizing under stringent conditions to the polynucleotide sequences of the present invention or to portions thereof.

The term hybridizing to a "portion of a polynucleotide sequence" refers to a polynucleotide which hybridizes to at least 12 nt, more preferably at least 18 nt, even more preferably at least 24 nt and especially to about 50 nt of a polynucleotide sequence of the present invention.

The present invention further provides isolated nucleic acid molecules comprising a polynucleotide sequences which is at least 95% identical, and preferably from 96% to 99% identical to the polynucleic acid sequence encoding the full length staufen polypeptides (i.e. 55 and 63 kDa hStau) or fragments and/or derivatives thereof. Methods to compare sequences and determine their homology/identity are well known in the art and exemplified herein.

As used herein, "chemical derivatives" is meant to cover additional chemical moieties not normally part of the subject matter of the invention. Such moieties could affect the physico-chemical characteristic

of the derivative (i.e. solubility, absorption, half life and the like, decrease of toxicity). Such moieties are exemplified in Remington's Pharmaceutical Sciences (1980). Methods of coupling these chemical-physical moieties to a polypeptide are well known in the art.

The term "allele" defines an alternative form of a gene which occupies a given locus on a chromosome.

As commonly known, a "mutation" is a detectable change in the genetic material which can be transmitted to a daughter cell. As well known, a mutation can be, for example, a detectable change in one or more deoxyribonucleotide. For example, nucleotides can be added, deleted, substituted for, inverted, or transposed to a new position. Spontaneous mutations and experimentally induced mutations exist. The result of a mutations of nucleic acid molecule is a mutant nucleic acid molecule. A mutant polypeptide can be encoded from this mutant nucleic acid molecule.

As used herein, the term "purified" refers to a molecule having been separated from a cellular component. Thus, for example, a "purified protein" has been purified to a level not found in nature. A "substantially pure" molecule is a molecule that is lacking in all other cellular components.

The term "isolated polypeptide" refers to a polypeptide removed from its natural environment. Non-limiting examples of isolated polypeptides include a polypeptide produced recombinantly in a host cell and partially or substantially purified polypeptides from such host cells. The polypeptides of the present invention comprise polypeptides encoded by the nucleic acid molecules of the present invention, as shown for example in Figure 1A, 1B, 1C, 1D and 1'. The present invention also

provides polypeptides comprising amino acids sequences which are at least 95% homologous, preferably from 96-99% homologous, even more preferably at least 95% identical and especially preferably from 96% to 99% identical to the full length staufen polypeptide sequence or fragments or derivatives thereof.

As used herein, the terms "molecule", "compound" or "ligand" are used interchangeably and broadly to refer to natural, synthetic or semi-synthetic molecules or compounds. The term "molecule" therefore denotes for examples chemicals, macromolecules, cell or tissue extracts (from plants or animals) and the like. Non-limiting examples of molecules include nucleic acid molecules, peptides, antibodies, carbohydrates and pharmaceutical agents. The agents can be selected and screened by a variety of means including random screening, rational selection and by rational design using for example protein or ligand modelling methods such as computer modelling. The terms "rationally selected" or "rationally designed" are meant to define compounds which have been chosen based on the configuration of the interaction domains of the present invention. As will be understood by the person of ordinary skill, macromolecules having non-naturally occurring modifications are also within the scope of the term "molecule". For example, peptidomimetics, well known in the pharmaceutical industry and generally referred to as peptide analogs can be generated by modelling as mentioned above. Similarly, in a preferred embodiment, the polypeptides of the present invention are modified to enhance their stability. It should be understood that in most cases this modification should not alter the biological activity of the interaction domain. The molecules identified in accordance with the teachings of the present invention have a therapeutic

value is diseases or conditions in which the physiology or homeastasis of the cell and/or tissue is compromised by a defect in in modulating gene expression and/or translation. Alternatively, the molecules identified in accordance with the teachings of the present invention find utility in the development of more efficient cell lines or cell extracts for translating mRNAs. Non-limiting examples of diseases and/or conditions in which the protein and/or nucleic acid molecules of the present invention find utility include cancer, apoptosis and aberrant proliferation of cells.

As used herein, agonists and antagonists of translation activity also include potentiators of known compounds with such agonist or antagonist properties. In one embodiment, agonists can be detected by contacting the indicator cell with a compound or mixture or library of molecules, for a fixed period of time, and then determining the effect of the compound on the cell.

The level of gene expression of the reporter gene (e.g. the level of luciferase, or β-gal, produced) within the treated cells can be compared to that of the reporter gene in the absence of the molecule(s). The difference between the levels of gene expression indicates whether the molecule(s) of interest agonizes the aforementioned interaction. The magnitude of the level of reporter gene product expressed (treated vs. untreated cells) provides a relative indication of the strength of that molecule(s) as an agonist. The same type of approach can also be used in the presence of an antagonist(s).

Alternatively, an indicator cell in accordance with the present invention can be used to identify antagonists. For example, the test molecule or molecules are incubated with the host cell in conjunction with one or more agonists held at a fixed concentration. An indication and

relative strength of the antagonistic properties of the molecule(s) can be provided by comparing the level of gene expression in the indicator cell in the presence of the agonist, in the absence of test molecules vs in the presence thereof. Of course, the antagonistic effect of a molecule can also be determined in the absence of agonist, simply by comparing the level of expression of the reporter gene product in the presence and absence of the test molecule(s).

It shall be understood that the *"in vivo"* experimental model can also be used to carry out an *"in vitro"* assay. For example, cellular extracts from the indicator cells can be prepared and used in one of the aforementioned "*in vitro*" tests (such as binding assays or *in vitro* translations).

As used herein the recitation "indicator cells" refers to cells wherein an interaction between staufen and dsRNA and/or staufen and tubulin for example is coupled to an identifiable or selectable phenotype or characteristic such that it provides an assessment of the interaction between these domains. Such indicator cells can be used in the screening assays of the present invention. In certain embodiments, the indicator cells have been engineered so as to express a chosen derivative, fragment, homolog, or mutant of staufen. The cells can be yeast cells or higher eukaryotic cells such as mammalian cells (WO 96/41169). In one particular embodiment, the indicator cell is a yeast cell harboring vectors enabling the use of the two hybrid system technology, as well known in the art (Ausubel et al., 1994, *supra*) and can be used to test a compound or a library thereof. In one embodiment, a reporter gene encoding a selectable marker or an assayable protein can be operably linked to a control element such that expression of the selectable marker

or assayable protein is dependent on the interaction of the a staufen domain with a binding partner (i.e. tubulin). Such an indicator cell could be used to rapidly screen at high-throughput a vast array of test molecules. In a particular embodiment, the reporter gene is luciferase or β-Gal.

As exemplified herein below in one embodiment, at least one staufen domain may be provided as a fusion protein. The design of constructs therefor and the expression and production of fusion proteins are exemplified herein (i.e. Example 2) and are well known in the art (Sambrook et al., 1989, *supra*; and Ausubel et al., 1994, *supra*). In a particular embodiment, both the binding partner of staufen and staufen are part of fusion proteins.

Non-limiting examples of such fusion proteins include a hemaglutinin A (HA) fusions and Gluthione-S-transferase (GST) fusions, HIS fusions, FLAG fusions, and Maltose binding protein (MBP) fusions. In certain embodiments, it might be beneficial to introduce a protease cleavage site between the two polypeptide sequences which have been fused. Such protease cleavage sites between two heterologously fused polypeptides are well known in the art.

In certain embodiments, it might also be beneficial to fuse the interaction domains of the present invention to signal peptide sequences enabling a secretion of the fusion protein from the host cell. Signal peptides from diverse organisms are well known in the art. Bacterial OmpA and yeast Suc2 are two non-limiting examples of proteins containing signal sequences. In certain embodiments, it might also be beneficial to introduce a linker (commonly known) between the interaction domain and the heterologous polypeptide portion. Such fusion protein find

utility in the assays of the present invention as well as for purification purposes, detection purposes and the like.

For certainty, the sequences and polypeptides useful to practice the invention include without being limited thereto mutants, homologs, subtypes, alleles and the like. It shall be understood that generally, the sequences of the present invention should encode a functional (albeit defective) interaction domain. It will be clear to the person of ordinary skill that whether an interaction domain of the present invention, variant, derivative, or fragment thereof retains its function in binding to its partner can be readily determined by using the teachings and assays of the present invention and the general teachings of the art.

As exemplified herein below, the interaction domains of the present invention can be modified, for example by *in vitro* mutagenesis, to dissect the structure-function relationship thereof and permit a better design and identification of modulating compounds. However, some derivative or analogs having lost their biological function of interacting with their respective interaction partner may still find utility, for example for raising antibodies. Such analogs or derivatives could be used for example to raise antibodies to the interaction domains of the present invention. These antibodies could be used for detection or purification purposes. In addition, these antibodies could also act as competitive or non-competitive inhibitor and be found to be modulators of staufen activity.

A host cell or indicator cell has been "transfected" by exogenous or heterologous DNA (e.g. a DNA construct) when such DNA has been introduced inside the cell. The transfecting DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the

genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transfecting DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transfected cell is one in which the transfecting DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transfecting DNA. Transfection methods are well known in the art (Sambrook et al., 1989, *supra*; Ausubel et al., 1994, *supra*). It will be understood that extracts from animal cells or mammalian cells for example could be used in certain embodiments, to compensate for the lack of certain factors in lower eukaryotic indicator cells.

In general, techniques for preparing antibodies (including monoclonal antibodies and hybridomas) and for detecting antigens using antibodies are well known in the art (Campbell, 1984, In "Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology", Elsevier Science Publisher, Amsterdam, The Netherlands) and in Harlow et al., 1988 (in: Antibody - A Laboratory Manual, CSH Laboratories). The present invention also provides polyclonal, monoclonal antibodies, or humanized versions thereof, chimeric antibodies and the like which inhibit or neutralize their respective interaction domains and/or are specific thereto.

The present invention also provides antisense nucleic acid molecules which can be used for example to decrease or abrogate the expression of staufen. An antisense nucleic acid molecule according to the present invention refers to a molecule capable of forming a stable

duplex or triplex with a portion of its targeted nucleic acid sequence (DNA or RNA). The use of antisense nucleic acid molecules and the design and modification of such molecules is well known in the art as described for example in WO 96/32966, WO 96/11266, WO 94/15646, WO 93/08845, and USP 5,593,974. Antisense nucleic acid molecules according to the present invention can be derived from the nucleic acid sequences and modified in accordance to well known methods. For example, some antisense molecules can be designed to be more resistant to degradation to increase their affinity to their targeted sequence, to affect their transport to chosen cell types or cell compartments, and/or to enhance their lipid solubility by using nucleotide analogs and/or substituting chosen chemical fragments thereof, as commonly known in the art.

From the specification and appended claims, the term therapeutic agent should be taken in a broad sense so as to also include a combination of at least two such therapeutic agents. Further, the DNA segments or proteins according to the present invention can be introduced into individuals in a number of ways. For example, erythropoietic cells can be isolated from the afflicted individual, transformed with a DNA construct according to the invention and reintroduced to the afflicted individual in a number of ways, including intravenous injection. Alternatively, the DNA construct can be administered directly to the afflicted individual, for example, by injection in the bone marrow. The DNA construct can also be delivered through a vehicle such as a liposome, or nanoerythrosome which can be designed to be targeted to a specific cell type, and engineered to be administered through different routes.

For administration to humans, the prescribing medical professional will ultimately determine the appropriate form and dosage for a given patient, and this can be expected to vary according to the chosen therapeutic regimen (i.e DNA construct, protein, cells), the response and condition of the patient as well as the severity of the disease.

Composition within the scope of the present invention should contain the active agent (i.e. fusion protein, nucleic acid, and molecule) in an amount effective to achieve an inhibitory effect on HIV and related viruses while avoiding adverse side effects. Typically, the nucleic acids in accordance with the present invention can be administered to mammals (i.e. humans) in doses ranging from 0.005 to 1 mg per kg of body weight per day of the mammal which is treated. Pharmaceutically acceptable preparations and salts of the active agent are within the scope of the present invention and are well known in the art (Remington's Pharmaceutical Science, 16th Ed., Mack Ed.). For the administration of polypeptides, antagonists, agonists and the like, the amount administered should be chosen so as to avoid adverse side effects. The dosage will be adapted by the clinician in accordance with conventional factors such as the extent of the disease and different parameters from the patient. Typically, 0.001 to 50 mg/kg/day will be administered to the mammal.

As used herein, "RNA viruses" is used broadly to cover retroviruses and non-retroviruses (such as Reovirus and poliovirus).

As used herein, HIV is used loosely to refer to HIV-1, HIV-2 and to SIV and related viruses.

The present invention is illustrated in further detail by the following non-limiting examples.

EXAMPLE 1

MOLECULAR CLONING AND SEQUENCING OF THE cDNAs

In order to clone a human *staufen* homologue, the GenBank database was searched with *Drosophila* dsRNA-binding domain sequences to find consensus sequences and eventually design degenerate oligonucleotide primers for RT-PCR. However, searching in the expressed sequence tags (EST) database identified a partial sequence, clone HFBDQ83 (GenBank accession number T06248), with high homology to the *Drosophila* sequence. This clone was purchased from the American Type Culture Collection and used as a probe to screen both human brain (Clontech) and foetal total mouse (a generous gift from A. Royal) cDNA libraries as described previously (Wickham and DesGroseillers, 1991). DNA from the isolated λGT10 clones was subcloned into a Bluescript™ vector (Stratagene). Double-stranded DNA was sequenced by the dideoxynucleotide method, according to Sequenase™ protocols (United States Biochemical Corp.).

EXAMPLE 2

CONSTRUCTION OF FUSION PROTEINS

The 1.2 kbp BamHI fragment of the human HFBDQ83 cDNA was subcloned in frame in either pQE32 (Qiagen) or pMAL-c (New England Biolabs) thus generating the protein fused to a hexahistidine tag or to the maltose-binding protein (MBP), respectively. The protein was expressed in bacteria by inducing with IPTG, as recommended by the manufacturer. Full-length and internal fragments of the mStau protein were PCR-amplified and cloned into pMal-c to produce fusion proteins with the maltose-binding protein. For the expression of the internal

domains, which do not contain an endogenous stop codon, the PCR fragments were cloned in a modified pMal-c vector (pMal-stop) in which stop codons were introduced at the HindIII site, by the ligation of the annealed complementary oligonucleotides 5'-AGCTTAATTAGCTGAC-3' and 5'-AGCTGTCAGCTAATTA-3'. The MBP/mSTAU fusion protein, containing the full-length mStau sequence, was generated by PCR amplification with Vent DNA polymerase (New England BioLabs), using the primer pair 5'-CCTGGATCCGAAAGTATAGCTTCTACCATTG-3' and 5'-TACATAAGCTTCTAGATGGCCAGAAAAGGTTCAGCA-3'. The resulting 1562 bp fragment was digested with HindIII and BamHI, and ligated in the pMal-c vector. The C-terminal fragment (mSTAU-C) was amplified with the primer pair 5'-GGATGAATCCTATTAGTAGACTT-GCAC-3' and 5'-TACATAAGCTTCTAGATGGCCAGAAAAGGTTCAG-CA-3', digested with HindIII and cloned in the EagI* and HndIII sites of pMal-c. EagI* was created by filling in the cohesive ends of EagI-digested pMal-c vector using the Klenow fragment of DNA polymerase I. This fusion vector was then digested with SacI and EcoRI and the resulting fragment was subcloned in the pMal-stop vector to generate the mSTAU-RBD3 construct. The mSTAU-TBD construct was prepared by PCR using the primer pair 5'-GCTCTAGATTCAAAGTTCCCCAGG-CGCAG-3' and 5'-TTTAAGCTTCTCAGAGGGTCTAGTGCGAG-3'; the product was digested with XbaI and HindIII and cloned in the pMal-stop vector. mSTAU-RBD2 and mSTAU-RBD1 were constructed by first amplifying a fragment using the primer pair 5'-CAATGTATAAGCCC-GTGGACCC-3' and 5'-AAAAAGCTTGTGCAAGTCTACTAATAGGATT-CATCC-3'. The resulting product was digested with HindIII and cloned in the EagI* and HindIII sites of the pMal-stop vector. This vector was then

used to purify the 398 bp PstI and HindIII fragment, which was subcloned in the pMAL-stop vector to generate the mSTAU-RBD2 construct. In the same way, the mSTAU-RBD1 vector was obtained by digestion with SmaI and StuI, followed by recircularization of the digestion product using T4 DNA ligase. The mSTAU-RBD4 was PCR amplified using the primer pair 5'-ATAGCCCGAGAGTTGTTG-3' and 5'-TACATAAGCTTCTAGATGGC-CAGAAAAGGTTCAGCA-3'. The resulting fragment was digested with HindIII and ligated in the pMal-stop vector at the StuI and HindIII sites. All the MBP/staufen fusion plasmids were transformed in the BL-21 E.coli strain. The fusion proteins were obtained after induction with 1mM IPTG for 2-3 hours. Cells were lysed in SDS-PAGE loading buffer for immediate use, or frozen at -80°C for storage.

EXAMPLE 3

ANTIBODY PRODUCTION AND WESTERN BLOTTING

For the production of antibodies, a large amount of the his/hStau fusion protein was purified on Ni-NTA resin (Qiagen), as recommended by the manufacturers, and injected into rabbits, as done previously (Aloyz and DesGroseillers, 1995). For western blotting, cells were lysed in 1% n-octylglucosid, 1 mM PMSF, 1 mg/ml aprotinin and 1 mg/ml pepstatin A in PBS. Protein extracts were quantified by the Bradford method (Bio-Rad), and similar amounts of proteins were separated on 10% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. Membranes were blocked for 30 min in TBS (Tris-buffered saline) plus 5% dry milk and incubated with primary antibodies in TBS plus 0.05% Tween™ for 1 hr at room temperature. Detection was accomplished by incubating the blots with

peroxydase-conjugated anti-rabbit immunoglobulin antibodies (Dimension Labs) followed by Supersignal™ Substrate (Pierce), as recommended by the manufacturer.

EXAMPLE 4

RNA-BINDING ASSAY

Bacterial extracts from IPTG-induced cultures were separated on 10% SDS-polyacrylamide gels and the proteins transferred onto nitrocellulose membranes. Membranes were incubated in the presence of [^{32}P]-labeled RNA probes in 50 mM NaCl, 10 mM MgCl2, 10 mM Hepes, pH 8.0, 0.1 mM EDTA, 1mM DTT, 0.25% milk, for 2 hr at room temperature, washed in the same buffer for 30 min, and exposed for autoradiography. For competition assays, an excess of cold homopolymers (Pharmacia) was added to the hybridization mixture along with the labeled probe. The 3'-UTR of *bicoid* cDNA (position 4016 to 4972) which was PCR-amplified from *Drosophila* genomic DNA, and subcloned in the bluescript™ vector, was transcribed using T7 RNA polymerase in the presence of [α-^{32}P]CTP. Synthetic RNAs (Pharmacia) were labeled with T4 polynucleotide kinase in the presence of [δ-^{32}P]ATP.

EXAMPLE 5

TUBULIN-BINDING ASSAY

Bacterial extracts from IPTG-induced cultures were separated on 10% SDS-polyacrylamide gels and the MBP-tagged proteins were transferred onto nitrocellulose membranes. Membranes were incubated in 10mM Tris, pH 8.0, 150 mM NaCl (TBS) and 1% Tween 20 for 45 min prior to an overnight overlay with 7 mg/ml tubulin

(Sigma) in TBS plus 0.2% Tween 20. Blots were washed several times in TBS plus 0.2% Tween 20, and then incubated with a mixture of mouse monoclonal anti-α- and anti-β-tubulin antibodies (ICN). Bound antibodies were detected with secondary peroxydase-conjugated anti-mouse immunoglobulin antibodies (Dimension Labs) and Sup
(Pierce), as stated previously. Separate assays we
actin and anti-actin antibodies (both from Sigma).

EXAMPLE 6

IMMUNOFLUORESCENCE

Hstau/HA and hStau/GFP were constructed by PCR-amplification of the full-length cDNA using the primer pair 5'-TACATGTCGACTTCCTGCCA/GGGCTGCGGG-3' and 5'-TACAATCTAGATTATCAGCGGCCGCACCTCCCACACACAGACAT -3'. The 3'-primer was synthesized with a NotI site just upstream from the stop codon allowing ligation of a NotI cassette containing either three copies of the HA-tag or the GFP sequence. The resulting fragment was cloned in Bluescript following digestion with SalI and XbaI. The KpnI/XbaI fragment was then subcloned in the pCDNA3/RSV vector (Jockers et al., 1996) and a NotI-cassette was introduced at the NotI site. For the TBD/GFP fusion protein, the TBD was PCR-amplified with oligonucleotides on each side of this region (5'-TACATAAGCTTAAGCCACCATGGTCAAAGTTCCCCAGGCGC-3' and 5'- TACAATCTAGAGCGGCCGCGCTCAGAGGGTCTAGTGCGAG-3'). The sense primer contained an ATG initiation codon and the Kozak consensus sequence, upstream from the TBD sequence. The anti-sense primer contained a Not1 site, just upstream from a stop codon. The

resulting fragment was digested with HindIII and XbaI and cloned into the pCDNA3/RSV vector. The GFP NotI-cassette was then introduced at the NotI site.

Mammalian cells were transiently transfected with the cDNAs by the calcium/phosphate precipitation technique, fixed in 4% paraformaldehyde in phosphate buffered-saline (PBS) for 25 min at room temperature and permeabilized with 0.3% Triton X-100 in PBS containing 0.1% BSA. The cells were then blocked with 1% BSA in PBS, 0.3% Triton X-100 and incubated with mouse anti-HA, rabbit anti-calreticulin or rabbit anti-calnexin antibodies for 1 hr at room temperature, as indicated. Cells were washed in permeabilization buffer and incubated with fluorescein-conjugated or Texas-Red-conjugated species-specific secondary antibodies (Jackson Immunoresearch Laboratories, West Grove, PA) in blocking buffer for 1 hr. GFP and GFP fusion proteins were detected by autofluorescence. Mounting was done in ImmunoFluor Mounting Medium (ICN). For the analysis of cytoskeleton-associated proteins, transfected cells were first extracted in 0.3% Triton X-100, 130 mM HEPES (pH 6.8), 10 mM EGTA, 20 mM MgSO4 for 5 min at 4°C, as previously described (Davis et al., 1987). They were then fixed in 4% paraformaldehyde in PBS and processed for immunofluorescence as described above. Cells were visualized by immunofluorescence using the 63X planApochromat objective of a Zeiss Axioskop fluorescence microscope.

Confocal microscopy was performed with the 60X Nikon Plan Apochromat objective of a dual channel BioRad 600 laser scanning confocal microscope equipped with a krypton/argon laser and the corresponding dichroid reflectors to distinguish fluorescein and Texas

Red labeling. No overlap was observed between the fluorescein and Texas Red channels. Confocal images were printed using a Polaroid TX1500 video printer.

EXAMPLE 7

MOLECULAR CLONING OF MAMMALIAN STAUFEN cDNAs

In order to understand the mechanism of mRNA transport in mammalian cells, the human and mouse *staufen* homologues was cloned. Thirteen overlapping human cDNAs, ranging in size between 0.8 and 2.5 kb, were isolated from a human central nervous system cDNA library, using the expressed sequence tag (EST) HFBDQ83 cDNA as a probe (Figure 1A). Purified human HeLa cell poly(A)+ RNAs were also reverse transcribed and PCR-amplified using different 5'-RACE protocols, allowing the cloning of the 5'-end of the transcript. Two different cDNAs of 3217 and 3506 nucleotides were identified from overlapping clones (see below). The presence of multiple transcripts in human cells was confirmed by RT-PCR experiments (not shown). One of the human cDNAs was then used to screen a foetal total mouse cDNA library under low stringency conditions, which led to the isolation of a full-length cDNA (mStau)(GB accession number: AF061942). The nucleic and amino acid sequences of mStau Is shown in Figure 1C. The human and mouse proteins are 90% identical (98% similarity), as shown in the alignment of the sequences thereof (Figure 1D).

Hybridization of a Human Multiple Tissues Northern Blot with a human cDNA reveals that hStau mRNA is found in every tested tissue (Figure 2A), unlike the *Drosophila staufen* gene which is exclusively expressed in oocytes and in the CNS at the larval stage (St

Johnston et al., 1991). The size of the cDNAs is close to that of the transcripts, which migrate on a Northern blot as an unresolved large band of around 3.6 kb.

EXAMPLE 8

A DIFFERENTIAL SPLICING EVENT GENERATES TWO HUMAN STAUFEN PROTEINS

Characterization of the human cDNAs revealed the presence of four types of transcripts which only differ by an insertion of 289 bp at position 324 (Figures 1A and 1B). Interestingly, this sequence introduces an ATG initiation codon upstream from the first one found in the short transcript (Figure 1A). This suggests that two putative proteins of 63 and 55 kDa may be translated, with one protein exhibiting an 81 amino acid extension at its N-terminal extremity, as compared to the other protein. Using anti-hStau antibodies in western blot experiments, two protein bands of around 63 and 55 kDa in human cell extracts were observed (Figure 2B). To determine whether the cDNAs could account for the presence of the two proteins, each of them was subcloned in an expression vector and expressed in mammalian cells. As seen in Figure 2C, each cDNA gives rise to a single overexpressed protein which perfectly comigrates with the endogenous proteins.

To determine whether these transcripts are the products of differential splicing, genomic DNA was PCR-amplified with primers located on each side of the insert. The resulting fragments were cloned, and their extremities sequenced. Comparison of the genomic and cDNA sequences demonstrated that the DNA insert is carried on a single exon,

and that typical splicing consensus sequences are present at each intron/exon junction (not shown).

Taken together, these results demonstrate that the human *staufen* gene produces two different transcripts by alternative splicing and exon skipping, and that the transcripts code for two highly homologous proteins which differ in their N-terminal extremities.

EXAMPLE 9

COMPARISON OF THE MAMMALIAN AND *DROSOPHILA* STAUFEN PROTEINS

The amino acid sequences of the mammalian proteins are similar to that of the *Drosophila* staufen protein and of the product of an uncharacterized ORF on the X chromosome of Caenorhabditis elegans (Figure 2D and Figure 1'). The overall structure and relative position of the full-length and short-RBDs are well conserved and high sequence identity is found between corresponding dsRBDs. This is highly significant since an alignment of the domains found in the members of the dsRNA-binding protein family shows an average of only 29% amino acid identity to one another (St Johnston et al., 1992). In addition, domains 1 and 4 in the human sequence, which are short domains when compared to the consensus, are nevertheless highly similar to the corresponding fly sequences, even in the region that extends far beyond the N-terminal side of the consensus sequence, suggesting that they must play an essential role in staufen function.

Mammalian Stau does not contain the first dsRNA-binding domain nor the long N-terminal sequence of the *Drosophila* protein which was shown to bind to *oskar* protein (Breitwieser

et al., 1996). In addition, a putative tubulin-binding domain located between the third and fourth dsRNA-binding domains of mammalian Stau is not found in the Drosophila protein, at least at the amino acid level. This region contains a stretch of 91 amino acids which show 25% amino acid identity (66% similarity) to a microtubule-binding domain of MAP1B (Zauner et al., 1992). It is meaningful that the sequence similarity covers the full microtubule-binding domain of MAP1B and that it is restricted to this domain.

EXAMPLE 10

THE HUMAN AND MOUSE STAUFEN PROTEINS BIND DOUBLE-STRANDED RNAs

As seen in Figures 2D and 1', mammalian Stau proteins contain multiple dsRNA-binding domains. In order to determine whether Stau binds RNAs, two bacterially-expressed fusion proteins were used in an RNA-binding assay, his/hStau and MBP/mStau. The fusion proteins were probed with in vitro-labeled *bicoid* mRNA, which is known to adopt an extensive secondary structure and to strongly bind to the *Drosophila* staufen protein, both in vivo and in vitro (St Johnston et al., 1992; Ferrandon et al., 1994). Both fusion proteins strongly bind this RNA. The binding is competed by an excess of cold poly(rI)-poly(rC), but not by poly(rI), poly(rC), poly(rA) or poly(U), nor by tRNA or dsDNA (for example, see Figure 3A), suggesting that mammalian Stau recognizes double-stranded structures in the RNA rather than a sequence-specific region. Both fusion proteins also directly bind labeled double-stranded RNAs and RNA/DNA hybrids, but not single-stranded RNA or DNA homopolymers (for example, see Figure 3). As controls, a his/NEP

(neutral endopeptidase) or MBP/aminopeptidase fusion proteins were also included on the blot; they did not bind any of these nucleic acids.

This demonstrates that both the human and mouse staufen proteins, regardless of the protein to which they are fused, are able to bind dsRNAs and RNA with extensive secondary structure, as reported for the *Drosophila* protein (St Johnston et al., 1992).

EXAMPLE 11

THE HUMAN AND MOUSE STAUFEN PROTEINS BIND TUBULIN IN VITRO

As described above, Stau contains a region which is similar to the microtubule-binding domain of MAP-1B. To determine whether mammalian Stau can bind tubulin, bacterially-expressed MBP/Stau fusion proteins were used in a tubulin-binding assay. As shown in figure 4, hStau binds tubulin in vitro. As a control, the MBP/aminopeptidase fusion protein was also included on the blot; it did not show any tubulin-binding capability. Under the same conditions, hStau cannot bind actin (Figure 4), which suggests that the binding of tubulin to staufen is specific. The same results were obtained with the MBP/mStau fusion protein (see Figure 5B, lane 2). Binding to mRNAs and microtubules are two of the characteristics expected of localizing proteins, making hStau and mStau very good candidates for mRNA transport and localization in mammals.

EXAMPLE 12

MOLECULAR MAPPING OF THE RNA- AND TUBULIN-BINDING DOMAINS

To determine which staufen domain(s) is involved in RNA and/or tubulin binding, the MBP/mStau fusion protein was used to construct a series of deletion mutants (Figure 5). The production and relative abundance of each fusion protein was first verified by Western blotting (not shown). Using the RNA-binding assay, it was demonstrated that both of the full-size dsRNA-binding domains (dsRBD2 and dsRBD3) are independently sufficient to bind *bicoid* RNA (Figure 5A). In contrast, the two short-domains (dsRBD1 and dsRBD4) were unable to bind dsRNA in this assay. It was also demonstrated that the C-terminal half of mStau is able to bind tubulin (Figure 5B, lane 4). More specifically, the region which is similar to the MAP1B-microtubule-binding domain is sufficient to bind tubulin (Figure 5B, lane 6). The faint bands (Figure 5B, lanes 3 and 5) were not reproducible.

These experiments confirm that the regions identified by sequence comparison as putative dsRNA- and tubulin-binding domains are biochemically functional.

EXAMPLE 13

STAUFEN IS ASSOCIATED WITH THE DETERGENT-INSOLUBLE FRACTION *IN VIVO*

The cellular distribution and cytoskeletal association of the two human Stau proteins in vivo was then addressed. To do so, the Green Fluorescent Protein (GFP) or an HA-tag were fused to the 63 and 55kDa hStau isoforms, respectively. Using confocal microscopy, it was

first shown that the two fusion proteins co-localize when co-expressed in mammalian cells (not shown). Then, it was shown that they are non-homogeneously distributed throughout the cytoplasm and label numerous vesicular and tubular structures which concentrate in the perinuclear region (Figure 6A). Minimal staining was found in the nucleus. When the cells were treated with Triton X-100 prior to fixing, allowing soluble proteins to be separated from the cytoskeleton and cytoskeleton-associated proteins (Pachter, 1992), the tubulovesicular labeling was still present, demonstrating that hStau is associated with the detergent-insoluble material in vivo (Figure 6B). Labeled structures were also present in cell processes, suggesting that Stau may target mRNAs to peripheral ER elements. The same results were obtained following expression of the GFP/mStau protein (not shown). The association between hStau and the cytoskeletal-associated material was confirmed by in vitro cell fractionation in the presence of Triton X-100. In this assay, hStau partitioned mainly in the cytoskeleton-associated fractions, although a significant fraction was found in a soluble form, as judged by Western blotting (not shown).

To determine whether the tubulin-binding domain identified *in vitro* is truly involved in this function *in vivo*, mammalian cells were transfected with a cDNA coding for a fusion protein in which the minimal tubulin-binding domain was fused to GFP. In contrast to the full-length protein, the TBD/GFP fusion protein is randomly distributed in the cytoplasmic and nuclear domains of the cells (Figure 6C), as is the GFP protein used as a control (Figure 6D). This staining was completely extracted by the Triton X-100 treatment (not shown), suggesting that the minimal tubulin-binding domain found in vitro is not sufficient to render the

protein insoluble and form a stable association with the microtubule network and/or the cytoskeleton-associated material.

EXAMPLE 14

STAUFEN LOCALIZES TO THE ROUGH ENDOPLASMIC RETICULUM *IN VIVO*

Interestingly, the pattern of localization of Stau resembles that of the endoplasmic reticulum. To test a putative localization of Stau to the ER, mammalian cells were transfected with a cDNA coding for a fusion protein in which a HA-tag was introduced at the C-terminal end of the short hStau protein. The cells were then double-labeled transfected with anti-HA, to recognize hStau, and with anti-calreticulin or anti-calnexin, two markers of the ER. Using a confocal microscope, it was shown that hStau completely co-localizes with anti-calreticulin, although HA-staining appears to be absent in some parts of the ER, in particular around the nucleus (Figure 7A-C). To confirm these results, the co-localization of staufen and calnexin, a specific marker for the RER (Hochstenback et al., 1992)(Figure 7D-F) was examined. The patterns of staining obtained with anti-hStau and anti-calnexin were identical, demonstrating that hStau co-localizes exclusively with the RER.

EXAMPLE 15

IMPLICATION OF STAUFEN IN mRNA TRANSPORT AND LOCALIZATION

The transport and localization of specific mRNAs have important functions in cell physiology. For example, mRNA targeting plays

a key role in the formation of cytoskeletal filaments and in the establishment of morphogenetic gradients (St Johnston, 1995). However, the nature of the ribonucleoprotein complexes as well as the mechanisms involved in these processes are still largely uncharacterized. Herein, a novel RNA-binding protein which localizes to the rough endoplasmic reticulum in mammalian cells has been described. Although its precise role is still unclear, its biochemical and molecular properties strongly suggest that it is involved in mRNA transport and/or localization. Consistent with such a role, we recently demonstrated that hStau is involved in RNA virus encapsidation and more particularly in HIV-1 genomic RNA encapsidation (see below). Similarly, a mammalian staufen homologue was recently shown to be involved in the polarized transport of mRNAs in hippocampal neurons (Kiebler et al., submitted).

EXAMPLE 16

STRUCTURE/FUNCTION OF STAUFEN

As is the case for all members of the dsRNA-binding protein family (St Johnston, 1995), it was observed that mammalian staufen can bind any dsRNA or RNAs forming extensive secondary structures in vitro, regardless of its primary sequence, as well as RNA/DNA hybrids. The latter adopt a conformation that is more closely related to that of dsRNA than dsDNA, which probably explains why they can bind to staufen. The fact that the full-length Stau protein, as observed with single dsRBD, binds to any dsRNA in vitro, suggests that the correspondence between the position of the dsRNA-binding domains and the arrangement of double-stranded stems in the folded RNAs may not be sufficient for specificity; post-translational modifications and/or

essential co-factors capable of forming complex ribonucleoprotein structures along with mRNA molecules, could be necessary to discriminate between different RNA secondary structures. Packaging of mRNAs into ribonucleoprotein complexes (Ainger et al., 1993; Ferrandon et al., 1994; Forristall et al., 1995; Knowles et al., 1996), the intermolecular dimerization of the localization signal of *bicoid* mRNA (Ferrandon et al., 1997) and the involvement of untranslatable hnRNAs in mRNA transport (Tiedge et al., 1991; Tiedge et al., 1993; Kloc and Eskin, 1994), are consistent with this interpretation. Until now, specific mRNA/staufen interactions were only shown in vivo after injection of different RNAs into *Drosophila* embryos, but the mechanisms underlying the specificity are not known (Ferrandon et al., 1994). Since specific RNA binding cannot be obtained in vitro, it precludes the use of classic techniques to isolate and identify relevant RNAs which would bind staufen *in vivo*. Cross-linking of mRNA to staufen in vivo, and isolation of the resulting complexes will be necessary to identify the nature of bound RNAs.

Regardless of their limitations, the *in vitro* assays did allow a mapping of the molecular determinants which are necessary and sufficient to bind RNAs. The presence of two functional domains in the mammalian Stau contrasts with what has been reported for other members of the dsRNA-binding protein family, which contain multiple full-length dsRBDs, but only one that is biochemically functional (Gatignol et al., 1993; McCormack et al., 1994; Schmedt et al., 1995; Krovat and Jantsch, 1996). Interestingly, full-length dsRBDs incapable to bind dsRNA by themselves can do so when joined to another inactive full-length domain, suggesting that multiple domains present in a given protein

exhibit cooperative binding effect (Schmedt et al., 1995; Krovat and Jantsch, 1996). Whether the two mStau dsRNA-binding domains exhibit similar or different affinities is not yet clear. However, the identification of the molecular determinants of staufen necessary and sufficient for RNA binding open the way to a wide variety of utilities. Non-limiting examples include viral therapy and prevention, targeting of molecules (comprising staufen's incorporation domain) into virions and gene therapy. In this respect, the PCT publication of Cohen et al. WO 96/07741 is of relevance, as it identified a new means for targeting molecules into HIV virions. The teachings of WO 96/07741, including vpr/vpr fusion proteins, vpr/vpr recombinant proteins and nucleic acid molecules encoding same can be applied to the present invention, now that staufen has been identified as a RNA-virus targeting protein and more particularly as a HIV targeting protein.

Tubulin-binding domain was mapped to a region which is similar to a microtubule-binding domain of MAP1B. Although this region can efficiently bind tubulin in vitro, it is not sufficient to bring a TBD/GFP fusion protein to the microtubule network. Binding of Stau to microtubules *in vivo* may involve more than one molecular determinant or the proper localization and folding of the TBD in the full-length protein. Indeed, in our *in vitro* assay, the fusion protein which contains the C-terminal region in addition to the TBD binds tubulin more efficiently than does the TBD, alone, suggesting that this region may be necessary for binding to microtubules *in vivo*. Interestingly, the corresponding region of the *Drosophila* staufen protein was shown to bind inscutable (Li et al., 1997), a protein with ankyrin domains which is believed to associate with the cytoskeleton (Kraut and Campos-Ortega, 1996), suggesting that

corresponding regions of the mammalian and *Drosophila* proteins may have functional similarities. The characterization of the mammalian staufen can therefore provide a guidance for a broadering of the present teachings to lower eukaryotic staufen such as that of *Drosophila* and as of *C. elegans.*

Alternatively, binding may be weak and/or transitory in vivo, for example during the early steps of mRNA recruitment, during mRNA transport and/or at mitosis, as reported in *Drosophila* (Ferrandon et al., 1994; Pokrywka and Stephenson, 1995; St Johnston, 1995). These steps may be difficult to observe by immunofluorescence (Ferrandon et al., 1994), and/or be masked by the anchoring of the protein to the RER. These steps may nevertheless be necessary to allow efficient and flexible transport of RNA along the cytoskeleton. In *Drosophila*, there is no evidence that staufen directly binds to the microtubule network, although staufen-dependent mRNA transport was shown to rely on this structure (Pokrywka and Stephenson, 1995; St-Johnston, 1995). A similar conclusion was reached when binding of MAP1B to the microtubule network was studied (Zauner et al., 1992), suggesting that weak binding to the cytoskeleton may be a characteristic of proteins containing this type of tubulin-binding domain.

The present teachings demonstrate that Stau is anchored to the RER and that the putative TBD is not involved in this function. Indeed, preliminary results suggest that the binding of Stau to RER is carried out by one of the RNA-binding domains (data not shown). Similar domains in other members of the dsRNA-binding proteins were previously shown to be involved in protein dimerization and/or in protein/protein interactions (Cosentino et al., 1995; Benkirane et al.,

1997). This also suggests that different Stau molecular determinants are necessary for binding to tubulin and anchoring to the RER. This is consistent with previous observations in Xenopus and *Drosophila* that demonstrated that mRNA localization was likely to occur via successive steps involving different elements of the cytoskeleton and overlapping molecular determinants (St Johnston, 1995).

EXAMPLE 17

LOCALIZATION OF STAUFEN TO THE RER

When expressed in mammalian cells, Stau isoforms show a tubulovesicular pattern of localization which is found more abundantly in the perinuclear region. Stau is the first RNA-binding protein shown to be associated with the RER in mammals. No signal peptide or putative hydrophobic transmembrane domains are present in either the long or short staufen proteins, indicating that they are cytosolic proteins and not residents of the RER and that their association to the RER is likely to reflect their mRNA transport function. Two recent papers also suggest that mRNA transport may be linked to the endoplasmic reticulum or ER-like structures. In Xenopus oocytes, vera, a Vg1 mRNA binding protein, was shown to co-sediment with TRAPa, a protein associated with the protein translocation machinery of the ER. However, in contrast to Stau, vera/Vg1 complexes were found associated only with a small subdomain of the ER, which was of the smooth variety (Deshler et al, 1997). Similarly, in *Drosophila*, at least some steps in mRNA transport in nurse cells and oocytes seem to occur within ER-like cisternae (Wilsch-Bräuninger et al., 1997). As observed for the Vg1 mRNA/SER

interaction in Xenopus, this structure seems to exclude most ribosomes, suggesting that translation is not the major function of these associations.

Hstau and mStau represent new members of a large family of proteins involved in the transport and/or localization of mRNAs to different sub-cellular compartments and/or organelles. Stau, TRBP/Xlrbpa and Spnr were shown to co-localize with RER (see above), with ribosomes and heterogenous nuclear RNPs (Eckmann and Jantsch, 1997), and with the microtubular array of spermatids (Schumacher et al., 1995), respectively. The present results strongly suggest that staufen/mRNA ribonucleoprotein complexes are transported along the microtubule network and then anchored to the RER. It is well known that the ER is associated with the microtubule cytoskeleton (Terasaki et al., 1986). Therefore, a transient interaction between microtubules and Stau may facilitate the localization of Stau and the targeting of mRNA to the RER. One of the roles of Stau might be to transport and localize specific mRNAs to the RER, such as those coding for secreted or membrane proteins which have to be translocated to the RER. This would bring them in proximity to the signal recognition particles (SRP) and RER, thus facilitating translation and translocation. The presence of Stau in cell processes, in association with ER structures, may represent a first clue to understanding the role of many mRNAs coding for neuropeptides, receptors or ion channels which were found to be localized in neuronal processes (Steward, 1997). Stau may facilitate the transport of mRNAs to cell processes to ensure efficient local translation and translocation. In addition, the presence of multiple staufen-like proteins in mammals creates the possibility that different members of the family could target sub-classes of mRNAs to different sub-domains of the ER. This

phenomenon has been described before, and is thought to be the first step in the differential targeting of proteins in polarized cells (Okita et al., 1994).

The possibility that staufen plays additional roles in mammals is not excluded; Stau may first be linked to the RER for storage, then a subset of molecules may be recruited by specific mRNAs and/or cofactors to form ribonucleoprotein complexes that will be transported along microtubules toward their final destination. Consistent with this possibility is the presence of large amounts of Stau in the perinuclear region, where it may await the nucleo-cytoplasmic transport of mRNAs. Alternatively, Stau may play key roles in the regulation of translation of localized mRNAs. The fact that *Drosophila* staufen is essential for the translation of oskar mRNA, once it is localized at the posterior pole, is consistent with this hypothesis (Kim-Ha et al., 1995). Characterization of mRNAs and putative co-factors which bind to staufen will be necessary to understand the process.

In vertebrates, the mechanisms which underly the transport of mRNAs have not yet been deciphered. Characterization of the RNAs and proteins involved in transport and localization is particularly important since understanding the mechanisms responsible for the transport of mRNAs is fundamental for learning more on the development of polarity in cells, both during mammalian development and in somatic cells, at a time where RNA-based gene therapy is being considered as a possible approach to cure different disorders.

The present invention therefore opens the way to a development of better strategies for RNA-based gene therapy.

EXAMPLE 18

STAUFEN IS INCORPORATED INTO HIV-1 VIRIONS

In order to assess the functional significance of the dsRNA-binding activity of *staufen* in mammalian cells, the possibility of its binding to the TAR sequence in the HIV-1 RNA leader was investigated (Fig. 8A). Its association with HIV-1 was further investigated by determining whether hStau was incorporated into HIV-1 particles, a possible result of its double-stranded RNA binding capacity. Indeed, using a polyclonal antiserum generated to highly purified recombinant hStau, the corresponding 55 and 63 kDa species (5) of *staufen* were identified in purified viral preparations of laboratory strains of HIV-1 HxBc2 (HxBru, HxBH10) and pNL4.3, and in vesicular stomatitis virus G (VSVG) envelope pseudotyped HIV-1 particles (data not shown and Fig. 8) generated in human T lymphocyte (MT4 and Jurkat) or epithelial (293T) cell lines (data not shown).

To further substantiate hStau virion incorporation, sucrose gradient analyses were performed. First, microfiltered and ultracentrifuged HxBru virus was prepared in 293T cells. This cell type produces negligible amounts of contaminating microvesicles that contain cellular proteins (8). The virus was fractionated in a 20-60% sucrose gradient, and the presence of hStau in each fraction was evaluated by Western blot analysis. hStau was found to cosediment with reverse transcriptase (RT) activity, strongly indicating incorporation or strong association with viral particles (Fig. 8B). To further support virion incorporation, a subtilisin protease assay was performed on virus preparations (9). While envelope glycoprotein gp120 was completely degraded as expected after subtilisin treatment, viral proteins p24 and

p17 remained in large part protected since they are found within the virus (Fig. 8C). hStau also remained intact (Fig. 8C), though there appeared to be some degradation by subtilisin treatment. This same phenomenon was recently observed in virus generated in H9 and CEM cells where an actin isoform was shown to be incorporated within HIV-1 particles while some of the protein was also sensitive to subtilisin treatment (10).

Incorporation of hStau in two T-tropic viral clinical isolates minimally passaged in MT4 cells, and in three other retroviruses, HIV-2, murine leukemia virus (MLV) and Casitas brain ecotropic MLV (CasBr; 11) was then examined. All of these vector viruses incorporated hStau (Fig. 8D) suggesting a common functional role. Of note, hStau was also shown to be incorporated into a non retrovirus RNA virus, Reovirus (data not shown). Purified cell-free preparations of the DNA viruses, adenovirus, Epstein Barr virus (EBV) and human herpesvirus 6 (HHV-6) did not contain hStau. The presence of hStau was evaluated in concentrated cell-free and cesium chloride-banded preparations of Adenovirus (kindly provided by Dr. Bernard Massie, Biotechnology Research Institute, Montreal, Quebec), EBV and HHV-6 (both kindly provided by Drs. Ali Ahmad and José Menezes, Department of Microbiology and Immunology, University of Montreal). hStau was assessed by Western blot analysis: there were no detectable bands corresponding to hStau in up to 20 x 10^6 viral particles.

While hStau is incorporated into virions, the dsRNA- and TAR RNA-binding proteins TAR RNA-binding protein (TRBP), dsRNA-activated protein kinase (PKR) and Tat, are not detectable in purified preparations of HIV-1 (Fig. 8E). Taken together, these data show

that the TAR-binding activity is not sufficient to enable virion incorporation.

Confocal laser scanning microscopy was employed to determine the precise localization of hStau in HIV-1-producing cells. pNL4.3 and a hemagglutinin (HA)-tagged hStau were coexpressed in 293T cells and p24 and hStau were visualized by Texas Red- and fluorescein-conjugated secondary antibodies, respectively, in indirect immunofluorecence analyses (Fig. 9). hStau showed a diffuse cytoplasmic staining (5) and a large proportion of hStau was found to be colocalized with p24 antigen at the cell periphery (Fig. 9C). This colocalization is suggestive that hStau is present at sites of virus assembly, consistent with its presence in virions.

EXAMPLE 19

CHARACTERIZATION OF MOLECULE DETERMINANTS, INVOLVED IN STAUFEN INCORPORATION INTO HIV-1

On the basis of hStau TAR RNA-binding and its virion incorporation, a role for hStau in virus assembly was investigated. It was therefore attempted to correlate genomic RNA encapsidation with hStau incorporation in HIV-1. Transfection of wildtype provirus DNAs yields virus particles containing comparable amounts of hStau (Fig. 10, lanes 1 & 2). Genomic RNA encapsidation in HIV-1 is primarily mediated through the association of the packaging (*psi*) domain in the 5' leader sequence with the nucleocapsid (NC) protein (13). Therefore, an HIV-1 molecular clone HxBru in which the ^{28}Cys and ^{49}Cys of NC were mutated to Ser (^{28}C/^{49}C-S; 14) was initially tested. It was found that hStau incorporation was drastically reduced in these virus preparations (cf. Fig. 10, lane 3). Several other HIV-1 proviruses with NC mutations and deletions (15), and

a *psi* domain deletion mutant (16) were then tested, most of which generate noninfectious virus particles that are significantly impaired in RNA encapsidation. With the exception of the $^{36}C/^{39}C$-S NC mutant, transfection of all NC and *psi* mutant DNA proviral constructs generated virus particles that contained negligible amounts of hStau. Genomic RNA encapsidation was assessed in Northern blots and these analyses revealed that the *psi* and NC mutant constructs yielded virus with drastically reduced levels of genomic 9 kilobase pair (kb) RNA. In the $^{36}C/^{39}C$-S NC mutant virus preparation (Fig. 10, lane 5) hStau is present at approximately wildtype levels, and at the same time near wildtype levels of genomic RNA encapsidation are observed, consistent with several earlier observations (17). hStau incorporation into HIV-1 particles is thus strongly correlated with genomic RNA encapsidation. Consequently, hStau may indeed sort viral RNAs into a vicinity of an infected cell where Gag proteins are present, during assembly of virus particles. Alternatively, the data presented herein suggest that hStau incorporation is mediated through both the *psi* and NC domains; and with the recent structural characterization of NC-*psi* binding (18) it will be interesting to determine whether hStau is necessary for this conserved and critical association.

EXAMPLE 20

INCORPORATION OF STAUFEN INTO HIV-1 VIRIONS DECREASES THE INFECTIVITY THEREOF

Whatever the particular mechanism of incorporation of hStau into HIV-1, the present invention clearly identifies a new HIV-targeting molecule. The effects of incorporated hStau on the infectivity

of HIV-1 particles were investigated. hStau with pNL4.3 was overexpressed in 293T cells and a corresponding increase in hStau was found in purified virus preparations (Fig. 11A). Equal amounts of virus from pNL4.3- and pNL4.3/hStau-transfected cells were used to infect HeLa-CD4-βGal (MAGI; 19) and BF-24 (20) indicator cells. Both infectivity assays indicated that an excess amount of hStau in HIV-1 particles has a marked negative effect on virus infectivity [4- and a 6.7-fold decrease in MAGI and BF-24 assays, respectively; Fig. 4B & C]. These data further support the contention that hStau plays an integral role in virus assembly and can contribute to the infectious potential.

EXAMPLE 21

DISCUSSION AND IMPLICATIONS OF THE ROLE OF STAUFEN IN RNA ENCAPSIDATION

Because all cells examined until now express hStau, its virion incorporation is indicative of a late role in viral assembly. hStau's ability to bind double-stranded and structured RNAs may result in virion incorporation which would be consistent with a role in the sorting of retroviral genomic RNAs to sites of virus assembly. While the other TAR RNA- and dsRNA-binding proteins have important roles in HIV-1 gene expression and replication, hStau is shown here as the only member that is incorporated into virus particles. Moreover, hStau appears to be incorporated into several retroviruses as well as RNA viruses -and not DNA viruses- suggesting a common role for hStau in the assembly process of RNA viruses.

Overexpression of hStau leads to a marked increase in the amount of hStau in virus preparations (Fig. 11A). As a consequence,

virus infectivity is negatively affected (Fig. 11B & C). These results may be explained by steric hindrance or an inappropriate amount of encapsidated viral RNA. Nevertheless, the data herein presented demonstrate that an appropriate amount of incorporated hStau is required to generate infectious viral particles. Accordingly, our infection backcross experiments using MT4 and Jurkat cells show that the quantity of incorporated hStau is independent of the cell line, contrary to what was found for cellular proteins within the HIV-1 envelope. Briefly, backcross experiments were performed using MT4 and Jurkat T cells. 50 ng p24 pNL4.3 virus equivalents were used to infect Jurkat and MT4 cells. Cells were washed extensively and allowed to become productively infected. Virus was then harvested from each culture, purified, and the same amount was used to infect the other cell type. Virus was again harvested and hStau was evaluated in the all virus preparations by Western analysis using equal quantities of virus from each preparation. hStau levels per ng p24 were relatively constant in all virus preparations, in contrast to what was found for proteins embedded in the HIV-1 envelope [(cf. L. Bastiani, S. Laal, M. Kim, S. Zolla-Pazner, *J.Virol.* **71**, 3444 (1997)]. Based on the role of staufen for HIV infectivity, it will be interesting to see the effect of the expression of a staufen antisense on HIV-1 replication and /or morphogenesis. It is tempting to speculate that such an antisense expression (or the expression of an antibody directed against staufen) will reduce the infectivity of HIV. Based on the apparent role of staufen in RNA viruses in general, such an approach might also be beneficial for other RNA viruses.

TAR RNA-binding in HIV-1 has a critical role in transcription (22), but has also been shown to regulate viral gene

expression post-transcriptionally (23). All members of the dsRNA-binding protein family are associated with the translational machinery including *xlrbpa* which can bind to free ribosomal subunits and mRNAs in *Xenopus* oocytes (24), and PKR that was recently shown to be associated with 40*S* ribosomal subunits (25). Furthermore, TRBP can modulate PKR phosphorylation of eIF-2α to modulate HIV-1 gene expression (26). TRBP was also recently shown to interact with Tax of HTLV-1 (27) and this could modulate gene expression at transcriptional and/or post-transcriptional levels. Likewise, additional regulatory roles for hStau are expected to be uncovered. In support of this are preliminary studies that indicate that hStau can markedly relieve the TAR-mediated translational repression *in vitro* in reticulocyte lysates. Highly purified hStau (5) was incubated with a TAR-less RNA or a TAR-containing RNA generated by *in vitro* transcription of SP6CAT and SP6TARCAT plasmids [Parkin N.T. et al., *EMBO J.* 7, 2831 (1988)]. TAR dramatically reduced the amount of CAT protein produced *in vitro* translation as reported previously (*ibid.*). A dose-dependent derepression of CAT synthesis was observed when the TAR-CAT RNA was preincubated with recombinant hStau. There were no marked effects on CAT protein levels from the TAR- less RNA. This indicates that hStau has several functional parallels to its metazoan counterpart, and furthermore, its role in HIV-1 replication is likely to be multifaceted.

Herein, no attempt has been made to define the mechanism by which hStau is incorporated into HIV-1 particles but it is likely to require TAR-like and structured RNA domains characteristic of retrovirus leader sequences (29); although higher order structures may also be critical (18, 30). Virus incorporation of hStau may indeed be

mediated by both viral and cellular proteins. It has recently been determined however that the HIV-1 *vpr*, *env*, *vpu*, *pol* (protease, RT, integrase), and *nef* genes are dispensable for Stau incorporation (data not shown). To evaluate the role of HIV-1 genes in hStau incorporation, proviral constructs containing a mutated ATG initiation codon (*vpu*), frameshift sequence (*vpr*), premature stop codon (*nef*), and sequence deletions (EcoRI/EcoRI for *pol*; and a BglII/BglII for *env*) in HxBru were tested. In addition, VSV G pseudotyped HIV-1 particles incorporated hStau, thus indicating again that *env* is not necessary for hStau incorporation. However, both NC and the *psi* RNA domain are not only critical for genomic RNA encapsidation, but they also appear to mediate hStau incorporation. In light of the negative impact of hStau overexpression on viral infectivity, hStau may be a suitable target for an anti-HIV-1 strategy. Furthermore, in light of the demonstration that hStau is incorporated into other retroviruses as well as Reovirus, *staufen* may be a suitable target for anti RNA-virus therapy in general.

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Although the present invention has been described hereinabove by way of preferred embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.